Temporal Control of Colicin E1 Induction

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The expression of the gene encoding colicin E1, *cea*, was studied in *Escherichia coli* by using *cea-lacZ* gene fusions. Expression of the fusions showed the same characteristics as those of the wild-type *cea* gene: induction by treatments that damage DNA and regulation by the SOS response, sensitivity to catabolite repression, and a low basal level of expression, despite the presence of the fusion in a multicopy plasmid. Induction of expression by DNA-damaging treatments was found to differ from other genes involved in the SOS response (exemplified by *recA*), in that higher levels of DNA damage were required and expression occurred only after a pronounced delay. The delay in expression following an inducing treatment was more pronounced under conditions of catabolite repression, indicating that the cyclic AMP-cyclic AMP receptor protein complex may play a role in induction. These observations also suggest a biological rationale for the control of *cea* expression by the SOS response and the cyclic AMP-cyclic AMP receptor protein catabolite repression system.

Colicin E1 is a toxin that is active against *Escherichia coli*. Binding of this protein to a specific cell surface receptor leads to loss of the membrane potential and cell death (21). The gene for colicin E1, *cea*, is contained in the multicopy ColE1 plasmid (6). The *cea* gene is the first gene of an operon that also contains a gene, *kil*, whose product lyses cells, releasing colicin E1 into the medium (36, 39, 43, 48, 55). The control of colicin E1 synthesis is remarkable for several reasons. Induction of the *cea-kil* operon is lethal to the cell, due to the action of the *kil* gene product. The operon is normally tightly repressed, despite its presence in multiple copies in the cell. In an exponentially growing culture of cells carrying the ColE1 plasmid, synthesis is believed to be all or none, with a small fraction of the cells inducing spontaneously and releasing their colicin E1 into the medium (42). The majority of the cells are completely repressed and are immune to the effects of the colicin E1 in the medium due to another gene on the plasmid, *imm*. Although the medium contains only a limited amount of colicin E1 by this mechanism, it is sufficient to provide a selective advantage (1, 7) since a single colicin E1 molecule can kill an invading sensitive bacterium.

The repressor of the *cea-kil* operon is the LexA protein, which is encoded by the bacterial chromosome (11, 13, 18). LexA is also the repressor of the SOS response genes of the cell (22, 49). Following a DNA-damaging treatment, LexA is inactivated by proteolytic cleavage in a reaction that is promoted by the RecA protein, leading to induction of the expression of the SOS response genes as well as the *cea-kil* operon. There are two adjacent binding sites for LexA in the *cea* regulatory region (13), most likely accounting for the tight repression of the *cea-kil* operon. It would appear, however, that this regulatory arrangement would be disadvantageous to the cell in that *cea-kil* induction and cell death would be caused by DNA damage that the cell would normally be able to repair and then survive. In fact, it has been shown that the presence of the *cea-kil* operon does sensitize the cell to DNA-damaging treatments (36, 39, 43, 48, 55). In addition to regulation by the SOS response

system, *cea-kil* expression is regulated by catabolite repression (12, 20, 32, 40); and it has been reported that colicin E1 synthesis is stimulated by an effector of the stringent response, ppGpp (23), by anaerobiosis (32), and by supercoiling of the DNA (54).

To begin to understand the relation between these regulatory systems and *cea* expression, we used *cea-lacZ* gene fusions to quantitate synthesis. We found that there is a considerable delay between the inducing treatment and the onset of *cea* expression, and that this delay is increased under conditions of catabolite repression. These results suggest a mechanism by which the combination of catabolite repression and the SOS response is especially well suited for the regulation of the lethal expression of the *cea-kil* operon.

MATERIALS AND METHODS

Media and chemicals. L and M63 media (M63 salts supplemented with 0.2% glucose or glycerol, 0.4% Casamino Acids [Difco Laboratories, Detroit, Mich.], and 0.1 µg of thiamine per ml) were as described previously (41). Ampicillin and kanamycin were used at 150 and 25 µg/ml, respectively. To score *lacZ*⁺ colonies 5-bromo-4-chloro-3-indolyl-β-D-galactoside was used (41).

General methodology. Standard procedures were followed for P1 transduction, restriction enzyme digestion, ligation of DNA, and transformation of bacteria (25, 30, 41).

Bacteria and plasmids. The bacteria and plasmids used in this study are listed in Table 1. Plasmid pGE108 (Fig. 1) is a derivative of the ColE1 plasmid (6) that carries a *cea-lacZ* protein fusion. To construct pGE108, the region between the *Pst*I sites in the ColE1 plasmid (nucleotides 5282 to 6551 in the *cea* gene) was removed and replaced with a *Pst*I fragment from plasmid pMC1871 (4), which contains a truncated *lacZ* gene (containing the *lacZ* coding sequence from codon 9 to the end of the gene). This created a *cea-lacZ* protein fusion encoding a hybrid protein, with the 49 amino acids at its amino terminus from colicin E1 joined to β-galactosidase. In addition, a *Pst*I fragment encoding the kanamycin resistance gene from plasmid pUC71K (47) was inserted following the *lacZ* gene in pGE108. The sequence of the amino-terminal 15 amino acids of the hybrid protein produced by pGE108 was determined and found to correspond to that predicted from the DNA sequence of the *cea* gene (6, 53).

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TABLE 1. Bacteria and plasmids used in this study

Bacterium or plasmid	Genotype	Reference or source
Bacteria		
CA7901	CA8000 <i>crpO1</i>	J. Beckwith
CA8000	HfrH <i>thi</i>	2
CA8306	CA8000 $\Delta(cya)854$	2
GE13	GE2265 <i>malE::Tn10</i> <i>lexA3(Ind)</i>	This study
GE94	MC4100 $\Delta(gal-G[\lambda])$ <i>b2(\lambda)::[\Phi(recA-lacZ)I(Hyb)</i> <i>lacY⁺]</i>	50
GE152	GE2265 <i>lexA51(Def)</i>	52
GE642	GE2265 $\Delta(recA)1398$ <i>srl::Tn10</i>	This study
GE2130	GE2265 <i>recA441</i>	This study
GE2264	GE2265 <i>recA629(Cs)</i> <i>srl::Tn10</i>	This study
GE2265	<i>thr-1 leu-6 his-4(Oc)</i> <i>argE3 ilv(Ts) galk2</i> <i>rpsL1 supE mtl ara</i> <i>sulA11 $\Delta(argF-lac)U169$</i> <i>araD139 $\Delta(argF-lac)U169$</i> <i>rpsL150 relA1 fbbB5301</i> <i>ptsF25 deoC1</i>	52
MC4100		3
Plasmids		
pGE104	pBR322 Δtet $\Phi(cea-lacZ)2(Hyb)$	51
pGE108	ColE1 $\Phi(cea-lacZ)1(Hyb)$ <i>kan^r</i>	This study
pGE124	pBR322 Δtet $\Phi(cea-lacZ)3(Hyb)$	51

The construction of plasmids pGE104 and pGE124 (Fig. 1) has been described previously (51). Both plasmids are analogs of plasmid pBR322, in which the *tet* gene has been replaced by a gene fusion that is expressed in the same direction as *tet*. In plasmid pGE104, a *cea-lacZ* fusion at the *Pst*I site in *cea* is present, similar to that in pGE108, and a hybrid protein containing 49 amino acids from colicin E1 at its amino terminus is produced. The *cea* material from ColE1 in pGE104 starts at nucleotide 4226 of the ColE1 plasmid, 803 nucleotides 5' to the *cea* gene (i.e., the 5' end of the -35 hexamer in the promoter) and extends to the *Pst*I site (nucleotide 5287). In plasmid pGE124, the ColE1 material starts 106 nucleotides on the 5' side of *cea* (nucleotide 4923) and the gene fusion is at codon 3 of *cea* (nucleotide 5149).

DNA sequence determination. Appropriate DNA fragments were cloned into phage M13 mp11, and their sequences were determined by the chain-termination method described by Sanger et al. (37).

UV irradiation. Bacteria were grown in M63 supplemented medium to 2×10^7 to 4×10^7 cells per ml and then irradiated in glass petri dishes (diameter, 10 or 15 cm, depending on the volume) in a thin layer (≤ 1 mm) with gentle swirling. A UV germicidal lamp (15 W), emitting predominantly at 254 nm, was used; and the UV fluence was monitored with a UV meter (DM-254N; Spectroline). Irradiated bacteria were incubated with aeration in the dark.

Assay for β -galactosidase. The assay for β -galactosidase was performed on duplicate samples as described previously (30). Specific activities were calculated by using the following formula: specific activity = $1,000 \times (OD_{420} - 1.75 \times OD_{550}) / (OD_{600} \times T \times V)$, where OD is optical density at the indicated wavelength (in nanometers), *V* is the volume of culture present in a 1-ml assay (typically 0.05 to 0.2 ml), and

T is the length of the assay (in minutes). On induction, the induction ratio was calculated as the ratio of the enzymatic activity following induction to that present in the uninduced state.

RESULTS

Regulators of *cea* expression. To monitor *cea* expression, a *cea-lacZ* protein fusion was used. This fusion contained the 5' end of the *cea* gene, from its regulatory region to codon 49, fused to codon 8 of *lacZ*. In most experiments this fusion was present in one of two plasmids. In plasmid pGE108, the *cea-lacZ* hybrid gene replaced the *cea* gene in the ColE1 plasmid, which is its natural carrier. In plasmid pGE104, the gene fusion, as well as the 803 base pairs 5' to *cea* in ColE1, replaced the tetracycline resistance gene in plasmid pBR322. In some experiments plasmid pGE124 was used. This plasmid was similar to pGE104, except that the fusion to *lacZ* was at codon 3 of *cea*, and only the 106 base pairs 5' to *cea* were present.

The expression of the *cea* gene was quantitated under several different conditions. When the fusion was located in the multicopy ColE1 plasmid, the resulting pGE108 plasmid showed a low basal level of β -galactosidase expression (Table 2). This basal level was constant during exponential

FIG. 1. The *cea-lacZ* fusions used in this study. (A) Arrangement of relevant genes in the ColE1 plasmid. In plasmid pGE108, the material between the two *Pst*I sites was replaced with a truncated *lacZ* gene and a gene conferring kanamycin resistance, as shown. In plasmid pGE104 the region from the *Sau*3A site in the *exc-2* gene to the *Pst*I site at position 5282 was cloned into a pBR322 derivative, and a *lacZ* fusion was constructed at the *Pst*I site (B). In plasmid pGE124 a similar clone was constructed by using a fragment from the site of a $\gamma\delta$ insertion to the *Sst*II site at the beginning of *cea* (C). In the process of this construction, the *Sst*II site was converted to a *Bam*HI site. Abbreviations: ATG, translation start for colicin E1; P1, transcription start for colicin E1; δ , end of the $\gamma\delta$ insertion element; B, *Bam*HI site; P, *Pst*I site; R, *Eco*RI site; S, *Sal*I site; S2, *Sst*II site; X, *Sma*I site.

TABLE 2. Regulation of *cea* expression by the SOS response^a

Plasmid	Host	Treatment	β-Galactosidase activity	
			Basal level	Induced level ^b
pGE108	Wild type	UV	50	2,375 (48)
pGE108	Wild type	Mitomycin C	29	1,960 (68)
pGE104	Wild type	Mitomycin C	130	3,200 (25)
pGE104	<i>recA</i> deletion	Mitomycin C	5	4 (0.8)
pGE104	<i>lexA</i> (Ind mutant)	Mitomycin C	100	84 (0.8)
pGE104	<i>lexA</i> (Def)	Mitomycin C	5,540	5,260 (0.9)

^a Plasmid pGE108 was carried by the wild-type strain GE2265, which was grown in M63 medium supplemented with glycerol and assayed at 3 h after induction by UV irradiation (35 J/m²) or by the addition of mitomycin C (1 μg/ml). Plasmid pGE104 was carried by strains GE2265, GE642, GE13, or GE152, which were grown in L broth and assayed at 2 h after the addition of mitomycin C (1 μg/ml).

^b Induction ratios are given in parentheses.

growth but increased severalfold at the stationary phase (data not shown). Expression from this plasmid was highly inducible by treatment with DNA-damaging agents such as UV radiation or mitomycin C (Table 2), as was expected for control by the SOS response. These values are comparable to those obtained by direct measurement of colicin E1 production.

When the fusion was located in a derivative of plasmid pBR322, which has a higher copy number than ColE1, the resulting pGE104 plasmid showed a higher basal level than that observed with pGE108. At least a part of this increase in basal expression was likely due to the higher copy number of this plasmid, although other factors may have been involved, such as partial titration of the LexA repressor or differences in superhelix density. The induction ratio observed for *cea* expression from pGE104, while slightly lower than that from pGE108, usually represented more than a 10-fold increase in expression, indicating that repression by LexA was still significant. Expression of *cea* in pGE104 was regulated by the *recA* and *lexA* products (Table 2), as was expression in pGE108 (data not shown). Induction was prevented in a *lexA*(Ind⁻) mutant, whose repressor could not be inactivated, and in a *recA* deletion mutant that could not inactivate the LexA repressor. These mutations, especially the *recA* deletion, also reduced the basal level of expression from pGE104. As expected, high-level constitutive expression of

TABLE 4. Regulation of *cea* expression by the cAMP-CRP system: effect of carbon source

Plasmid	Carbon source	β-Galactosidase activity		Optimum dose (J/m ²)	Lag time (min)
		Basal level	Induced level ^b		
pGE108	Glycerol	24	820 (34)	50	20
	Glucose	10	240 (24)		
pGE104	Glycerol	240	4,320 (18)	35	20
	Glucose	232	1,390 (6)		
pGE124	Glycerol	700	7,400 (11)	35	20
	Glucose	460	2,200 (5)		

^a MC4100 carried the indicated plasmids and was grown in M63 medium supplemented with glucose or glycerol (0.2%). β-Galactosidase activity was assayed 3 h after irradiation to determine the dose response and induced level. The lag time was determined from a time course following irradiation with 5 J/m². Each value is the mean of two different experiments.

^b Induction ratios are given in parentheses.

the fusion was observed in a *lexA*(Def) mutant that lacked the LexA repressor. These observations indicate that the *cea-lacZ* fusion in pGE104 is properly regulated by the SOS response, despite the differences between this plasmid and ColE1.

Expression of the fusion was also affected by the cyclic AMP (cAMP)-cAMP receptor protein (CRP) catabolite repression system. Expression from pGE108 was reduced in *cya* and *crp* mutant hosts, and the effect of the *cya* mutation could be reversed by the addition of cAMP (Table 3). Similar results were obtained with plasmid pGE104 (data not shown). Expression from either pGE108 or pGE104 was also reduced when glucose was present in the medium (Table 4), showing the expected dependence on carbon source.

Delay in *cea* induction following DNA-damaging treatments. A significant difference in the kinetics and dose response for *cea* induction was observed when compared with the induction of the *recA* gene (Fig. 2). For *recA*, whose synthesis is repressed by the LexA protein, expression was fully induced without significant delay after low to moderate UV doses (4 and 25 J/m²), which is behavior that is characteristic of other

TABLE 3. Regulation of *cea* expression by the cAMP-CRP system: effect of *cya* and *crp* mutants^a

Bacterium	cAMP	β-Galactosidase activity	
		Basal level	Induced level ^b
CA8000 (wild type)	–	47	7,800 (165)
	+	63	7,000 (111)
CA8306 (<i>cya</i>)	–	30	1,500 (50)
	+	42	7,400 (176)
CA7901 (<i>crp</i>)	–	30	350 (12)
	+	28	350 (12)

^a Bacteria carrying plasmid pGE108 were grown in M63 medium supplemented with glycerol at 37°C. Mitomycin C (0.2 μg/ml) and cAMP (1 mM) were added, and β-galactosidase activity was determined after 2.5 h of incubation.

^b Induction ratios are given in parentheses.

FIG. 2. UV induction of *recA-lacZ* and *cea-lacZ* fusions. Bacteria carrying a *recA-lacZ* fusion (GE94; ●) or a *cea-lacZ* fusion [MC4100 (pGE108); ○] were grown in M63 medium supplemented with glycerol. Each culture was irradiated with UV doses of 4, 25, and 100 J/m² (as indicated on the curves) at time zero; and β-galactosidase (β-GAL) specific activity (SP. ACT.) was determined.

FIG. 3. *cea-lacZ* induction in a *recA441* mutant. Strain GE2130 (*recA441*) carrying plasmid pGE104 (Δ) was grown at 30°C in M63 medium supplemented with glycerol and shifted to 43°C at time zero. Strain GE2265 (*recA*⁺) carrying plasmid pGE104 ($\bullet$) was grown similarly but was also irradiated with 24 J/m² at the time of the temperature shift. Each value is the mean of two experiments. β -GAL, β -Galactosidase; SP. ACT., specific activity.

genes induced during the SOS response (27). In contrast, *cea* was only slightly induced at the low dose, while its induction exhibited a pronounced delay at the moderate dose. At moderate to high UV doses (25 and 100 J/m²) the rate of *recA* expression declined, most likely because the damage impaired the synthetic capacity of the cell. Induction lasted longer under these conditions, however, presumably because complete repair of the increased amount of damage took longer (50). Similar effects were seen on *cea* expression at these doses. In control experiments, the presence of the ColE1 plasmid did not affect the induction of the *recA* gene (data not shown). Thus, the requirement for higher doses for *cea* induction and the delay in induction seen for *cea* expression is behavior that is specific to the *cea* gene. These effects do not result from a general modification of the SOS response caused by the presence of the ColE1 plasmid.

Induction of *cea* in *recA* mutants. UV irradiation is known to reduce the protein synthetic capacity of a cell, and this could account for the delay in *cea* expression after irradiation. Although irradiation only significantly affected *recA* expression at the highest dose used, it is possible that *cea* gene expression was more sensitive to these physiological changes. To test this, *cea* induction was followed in a *recA441* mutant (5). In this mutant the RecA protein became

FIG. 4. *cea-lacZ* induction in a cold-sensitive *recA* mutant. The *recA629* mutant GE2264 carrying plasmid pGE108 was grown at 28°C in M63 medium supplemented with glycerol. UV irradiation was performed at time zero with a dose of 15 J/m². Cultures were shifted to 37°C at 0, 30, 60, and 90 min, as indicated by the arrows. Symbols: $\bullet$, not shifted to 37°C; $\circ$, not irradiated but grown at 37°C. β -GAL, β -Galactosidase; SP. ACT., specific activity.

FIG. 5. UV dose-response curve. MC4100(pGE108) was grown in supplemented M63 medium containing either glycerol (A) or glucose (B). Following UV irradiation, β -galactosidase (β -GAL) specific activity (SP. ACT.) was determined after 1.5 h (open symbols) or 3 h (closed symbols) of incubation. Each value is the mean of two experiments.

activated to promote LexA cleavage at high temperatures without any DNA-damaging treatment; thus, the SOS response can be induced by a temperature shift without any deleterious treatment to the cell. Temperature induction of *cea* expression in the *recA441* mutant showed a similar delay (about 20 min), as did UV induction in a related *recA*⁺ strain (Fig. 3). This delay following temperature induction is specific to the *cea* gene, since temperature induction of *recA* gene expression in this mutant occurs nearly immediately (B. Salles, unpublished data). It thus appears that the lag in induction is not due to the general reduction in protein synthesis caused by DNA-damaging treatments.

Induction of *cea* expression was also studied following temperature shifts in a *recA629* mutant, a strain that produces a cold-sensitive RecA protein (Fig. 4). At 28°C in this strain, when the RecA protein was not active, a low basal level of *cea* expression was observed (about 4 units) that was not significantly induced by UV irradiation. At 37°C *cea* expression was inducible by UV irradiation and showed the characteristic lag of about 30 min. When cells were grown and irradiated at 28°C and then incubated at 28°C for various periods before they were shifted to 37°C, induction of *cea* expression always occurred about 30 min after the shift to a high temperature, regardless of how long after irradiation the shift occurred. Thus, when cells were shifted to a high temperature 90 min after they had been irradiated, *cea* expression still exhibited a lag of about 30 min. Results of these experiments further demonstrate the requirement for the RecA protein in *cea* induction and show that the process that leads to delayed expression of the *cea* gene does not start until the RecA protein becomes activated to promote cleavage of the LexA repressor.

Effect of catabolite repression on the characteristics of *cea* induction. To determine what role catabolite repression might play, the induction of *cea* expression was compared in media containing either glucose or glycerol as the carbon source. There was a dramatic difference in the characteristics of induction between these two conditions (Fig. 5 and Table 4). In glycerol medium, *cea* expression from plasmid pGE108 (measured 3 h after induction) increased with inducing UV doses up to 50 J/m² and then decreased at higher doses because of cell death (Fig. 5A). Except at low doses, the extent of induction was higher at 3 h than it was at 90 min after induction, with a maximum value of from 10- to 30-fold over the basal level of expression. In contrast, expression in

medium supplemented with glucose was reduced and delayed. The basal level of expression in medium supplemented with glucose was equal to or less than that observed in glycerol, and the extent of induction at 3 h was reduced (Table 4), although at later times the induction ratio approached that seen in medium supplemented with glycerol (data not shown). There was almost no induction in medium supplemented with glucose at 90 min after the inducing treatment, and maximum induction required a UV dose of at least 75 J/m² (Fig. 5B). A slower growth rate in medium supplemented with glucose than in that supplemented with glycerol was unexpectedly observed with these strains, and this may partially account for these differences. In other experiments in which growth rate was not a factor, however, qualitatively similar results were observed. Thus, it appears that catabolite repression decreases the rate of *cea* expression and increases the lag in induction.

Comparison of different *cea-lacZ* fusions. As noted above, the two plasmids used in the previous experiments, pGE108 and pGE104, contained essentially identical *cea-lacZ* fusions but differed in the plasmid backbone carrying the fusion. These two plasmids showed the same qualitative behavior with respect to regulation by the SOS response and catabolite repression and the delay in induction following DNA-damaging treatments. Plasmid pGE104 had the higher copy number of the two plasmids; and this may have led to less complete repression by LexA, resulting in the higher basal level, lower induction ratio, and lower UV dose required for maximal induction observed for strains with this plasmid. Plasmid pGE124 had the same plasmid backbone as pGE104, but pGE124 had less *cea* material fused to *lacZ*. Strains carrying pGE124 showed higher basal and induced levels of β -galactosidase activity when compared with those carrying pGE104 (Table 4). This difference may reflect the fact that in pGE124 there are only three amino acids from colicin E1 fused to β -galactosidase, whereas there are 49 amino acids fused in pGE104. Thus, the specific enzymatic activity of the hybrid protein may be higher with the fusion in pGE124, and the higher level of activity observed may result from this rather than from an increase in *cea* expression. Consistent with this is the observation that the induction ratios for *cea* expression were similar in these two plasmids (Table 4). The behavior of plasmid pGE124 resembled that of pGE104 in other respects as well, including the effect of catabolite repression, optimum UV inducing dose, and delay in induction (Table 4). Thus, the regulatory sequences responsible for these phenomena can be localized to the *cea* region present in pGE124, namely, from 106 base pairs 5' to the *cea* promoter to codon 3 of the structural gene, a region of 227 base pairs.

DISCUSSION

By using fusions to *lacZ* we were able to quantitate expression of the *cea* gene. The *cea-lacZ* hybrids faithfully mimicked the expression of the *cea* gene in that they showed the previously reported regulation by the SOS response and catabolite repression. This regulation was not only observed when the fusion was in the ColE1 plasmid but also when it was in a plasmid derived from pBR322 or was present as a single copy in the *E. coli* chromosome (unpublished data). Thus, the sequence responsible for this behavior are at the 5' end of the *cea* gene and do not require other plasmid sequences.

Results of previous studies have indicated that the uninduced level of colicin E1 production is very low, despite the

fact that the gene for this protein resides on a multicopy plasmid. Moreover, this basal production is due to only a small fraction of the cells (42). We found induction ratios of 40- to 100-fold, which is consistent with the idea that the basal level is due to a small percentage of the cells that were spontaneously fully induced, while the rest of the cells showed little or no expression. In addition, in *recA* mutants the basal level decreased 20- to 30-fold with plasmid pGE104. Since the RecA protein is required for SOS induction, this result further supports the idea that spontaneous induction of the SOS response in a small percentage of the cells is responsible for the basal level of production. The level of expression in *recA* mutants is also a measure of the efficiency of repression. Thus, the *cea-lacZ* gene is nearly totally shut off in this multicopy plasmid. Surprisingly, the *lexA*(Ind) mutant showed only a slight reduction in basal level, although there should have been little induction of the SOS response in this mutant. The effect of the *lexA*(Ind) mutation on basal level was more pronounced on a single copy of the *cea-lacZ* fusion in the bacterial chromosome (unpublished data). The reason for this result is not known, but it could imply a poorer binding of the mutant LexA repressor to the tandem operator sites in *cea*. Alternatively, the RecA protein may have an effect on *cea* expression that is independent of the SOS response. The copy number of the ColE1 plasmid has been shown to be unaffected in a *recA* mutant, however (9).

The *cea* gene induces poorly at low doses of DNA damage, and expression occurs after a significant lag, even at optimal doses. A lag in the production of colicin E1 has been observed previously following induction by UV irradiation (19) or thermal induction in a *recA441* mutant (44). Maximum induction of *cea* occurs at a dose similar to that found for other SOS response genes; therefore, there does not appear to be a general shift of the dose-response curve to higher doses. Rather, the poor induction seen at low doses can be accounted for by the lag in expression. At low UV doses the low levels of DNA damage can be repaired relatively rapidly, before the *cea* induction lag period has ended and *cea* expression has started. When repair is completed, inactivation of LexA ceases and repression of *cea* can be reestablished before significant synthesis from *cea* has occurred. Thus, only when persistent induction of the SOS response occurs, that is, at high enough UV doses so that repair cannot be completed in a short period of time, does *cea* expression commence before LexA reestablishes repression. Inasmuch as induction of the *cea-kil* operon is lethal to the cell, the delay in induction may enable cells to repair low levels of damage without being killed by toxic plasmid products. In previous studies it was shown that induction by mitomycin C is deleterious to cells carrying the ColE1 plasmid, presumably because there is no opportunity to shut off the SOS response. The presence of the ColE1 plasmid, however, only caused a slight increase in UV sensitivity (two- to threefold decreased survival) for the UV doses employed in the present study (unpublished data). It is thus possible that a biological function for the delay in induction may be to give cells that have limited DNA damage, from which they would normally efficiently recover, an opportunity to repair their DNA and reestablish repression before lethal induction of the *cea-kil* operon occurs.

One factor that could contribute to the lag is the tight binding of the LexA repressor to the *cea* regulatory region. It has been suggested that the affinity of LexA for this region is at least fourfold greater than that for other SOS response genes, possibly because the two tandem binding sites allow

for cooperative binding (13). Because only unbound LexA is believed to be proteolytically inactivated, it is possible that even though most LexA is rapidly cleaved following DNA-damaging treatments, the lag reflects the time necessary for LexA to dissociate from the *cea* operators. An additional possibility is that an SOS-controlled factor must accumulate in the cell before *cea* can be expressed.

Our results confirm earlier observations that *cea* expression is subject to catabolite repression, which is dependent on cAMP and CRP (12, 22, 32, 40). In *cya* or *crp* mutant strains, both basal and induced levels were reduced but not eliminated. These effects reflect, in part, a decrease in the rate of synthesis in the mutant strains, confirming that one role for the cAMP-CRP system is to increase the rate of transcription (12). UV irradiation has been shown to cause transient catabolite repression of the *lac* operon and other genes, presumably because the DNA damage creates a situation of unbalanced growth (24). Thus, *cea* expression may be reduced during the period of catabolite repression following UV irradiation. In addition, since CRP can reduce polarity on the expression of distal genes in an operon (17, 45), this catabolite repression could serve to lower transcription of *kil*. Hence, the dependence on the cAMP-CRP system may be another mechanism to retard *cea-kil* expression. Comparison of *cea* expression in medium supplemented with glucose or glycerol suggests that catabolite repression increases the delay in expression following an inducing treatment. If the delay is primarily due to the tight binding of the LexA protein, this could mean that the cAMP-CRP complex plays a role in removing the tightly bound LexA protein from the *cea* regulatory region.

The production of colicin E1 has been interpreted as an example of biological altruism. The idea is that, in a natural environment, colicin E1 is produced by otherwise viable cells, and that this synthesis is suicidal because of the lethal effects of *cea-kil* operon expression (29). However, our results suggest an alternative interpretation of this phenomenon. Since the spontaneously induced level of colicin E1 production is sufficient to afford a selective advantage (1, 7), this may be the significant mode of production in a natural environment. Although the event that causes spontaneous induction of the SOS response is not known, it seems reasonable to assume that its dose response is similar to that seen with UV irradiation. Low amounts of this DNA damage are repairable and allow a high level of cell survival. Such low doses are not sufficient for *cea* induction. On the other hand, those cells with high levels of spontaneous damage have a severely reduced survival potential because of the damage. High doses also cause prolonged induction of the SOS response, leading to full expression of the *cea-kil* operon. The regulation of the *cea-kil* operon by the SOS response may thus limit the natural production of colicin E1 to those cells that have suffered a catastrophe and that will most likely not survive anyway. Colicin E1 regulation by the SOS response thus appears to be an example of biological economy rather than altruism. The fact that other toxins are regulated by the SOS response (8, 14–16, 26, 28, 31, 33, 35, 38, 46, 56) suggests that this mechanism may be of general evolutionary significance. It is also noteworthy that some of these toxin genes are regulated by catabolite repression.

Other factors, in addition to the LexA protein and the cAMP-CRP complex, may contribute to the characteristics of *cea* expression. Several different promoters in the *cea* regulatory region have been reported (10, 34), as have effects of anaerobiosis (32), the stringent response effector ppGpp (23), and DNA supercoiling (54). Analysis of *cea* expression

promises to provide further insights into these global regulatory mechanisms, as well as the regulation of toxin production.

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